sects and were fixed immediately in aqueous Bouin's fluid. The material was processed in the usual way and paraffin blocks were prepared. 6–8 μm thick sections were serially cut and stained with FF-technique of Ewen[9].

The effect of light on the neurosecretory activity was shown by the autoradiographic studies on grasshopper, wherein a higher degree of S^{35}-cysteine was incorporated into the brain neurosecretory cells of the long day insects, as compared with the short day insects[5]. Recently, the site of photoperiodic reception was believed to be the brain itself by some workers[2,3]. In the present study, a few significant changes were observed in the cerebral NSC after exposing the insects to illumination of longer duration. Under illumination, the NSC were found to be depleted of neurosecretory material (NSM) (figure 1). It is suggested that, due to prolonged exposure to illumination, the synthesis of NSM is checked. The NSC were found densely packed with NSM after keeping the insects in total darkness (figure 2). Due to darkness, the NSM remains undischarged and thus accumulates as a densely packed material in the perikarya of the NSC. If these insects are again exposed to illumination, the NSC are deplated of their colloids. Similar observations were made by Gundevia and Ramamurty[10], who reported that light plays a role in the process of neurosecretory material production and its release, because in darkness the cells were densely packed with NSM, while under illumination the cells themselves are depleted of their colloids but in the axons there was an abundance of the neurosecretory granules.

Starvation brought about the inhibition of neurosecretory activity in red cotton bug[6], and clumping of NSC in *Orthetrum chrysis*[11]. In the present investigation, deformity in the NSC and clumping of the NSM in the cells and axons were brought about by 15 days of starvation in *P. americana* (figure 3). Similar observations were made by Bassurmanova and Panov[4]. No change was observed in the neurosecretory activity of brain cells due to different diets as compared with controls. Presumably diet plays no significant role in the secretory activity of NSC of this insect. In all the control insects, the cerebral NSC showed uniformly distributed NSM in their perikarya (figure 4).

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Distribution of γ-aminobutyric acid (GABA) in the ganglia of *Aplysia kurodai*

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Summary. The concentration of GABA was determined in each ganglion of *Aplysia kurodai* by microassay. Highest concentration was observed in buccal ganglia.

GABA has been established as an inhibitory neurotransmitter at the crustacean neuromuscular junction[2–4] and there is now good evidence that the substance is the inhibitory neurotransmitter in vertebrate[5,6] as well as in invertebrate nervous systems[2–4]. Morphological and physiological properties of *Aplysia* ganglia have been investigated for many years. The relatively large size and ready accessibility of the neurons facilitate neurochemical investigation including the determination of putative neurotransmitters; acetylcholine[7], dopamine and serotonin[8,9], glutamate and glutamine[10] and their related enzymes in the individual neurons of the ganglia.

However too little attention has been paid to GABA in the *Aplysia* ganglia. Presence of GABA in the ganglia has not been determined[3], probably because of the technical limitation of GABA assay system in such a small sample as single ganglion or neuron. By the iontophoretic study, Gerschenfeld et al.[11] found that GABA has an exitatory on H-cells and an inhibitory action on D-cells of *Aplysia* nervous system.

In the present experiment, the concentration of GABA was determined in each ganglion of *Aplysia kurodai*, by a sensitive method which combines the GABase system of Scott and Jacoby[12] with the technique of enzymatic cycling of NADPH of Lowry et al.[13]. This method enabled us to measure GABA in the order of 10^{-12} moles.

Aplysia kurodai (220–270 g b.wt) were collected at the Miura Peninsula, Kanagawa Prefecture, in January and February 1974. They were kept in a sea-water tank at

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Distribution of GABA in *Aplysia* ganglia

Ganglion	Animal number A15	A16	A17	A18	A24	Mean ± SD
Abdominal	0.30	0.72	0.75	0.48	0.34	0.51 ± 0.21
L-pedal	2.66	1.47	1.29	2.41	0.94	1.75 ± 0.74
R-pedal	2.40	1.70	2.48	5.09	1.57	2.65 ± 1.42
L-pleural	1.46	1.52	1.49	1.73	1.11	1.46 ± 0.22
R-pleural	1.08	1.44	2.58	2.09	1.53	1.74 ± 0.59
L-buccal	2.03	5.33	4.01	3.25	3.04	3.53 ± 1.23
R-buccal	3.97	5.90	6.16	6.14	3.36	5.11 ± 1.37
Cervical	2.39	1.72	2.22	3.31	2.45	2.42 ± 0.58
Connective nerve	0.53	1.48	1.03	1.50	0.70	1.05 ± 0.44
Buccal muscle	–	–	–	–	–	0.04 ± 0.03*

GAGA contents are shown as mmoles/kg protein. *Buccal muscle was dissected out from 3 other animals.

15 °C and fed with dried sea weed (*Porphyra* sp.) for 1–4 weeks before the experiments. Ganglia were dissected from the animals without anaesthesia using fine ophthalmological scissors and transferred to a dish containing filtered sea water at 2–8 °C. The connective tissue and sheaths were removed with a special care not to damage their neurons. Each ganglion was homogenized with a glass homogenizer containing 50–200 µl of 0.01 N HCl at 4 °C. The homogenates were centrifuged at 1000 × g for 15 min at low temperature. Resulting supernatants were stored at −20 °C until the assay of GABA. To the precipitates 200–400 µl of 0.5 N NaOH was added and they were dissolved and kept −20 °C for the protein determination.

An aliquot (3.8 µl) of the supernatants was transferred to the oil well[12] making small droplets and heated at 60 °C for 10 min to destroy NADPH in the extracts. After heating 4 µl of GABA assay reaction mixture containing 0.2 M tris-HCl buffer (pH 8.9), 10 mM α-ketoglutarate, 0.5 mM NADP, 0.01% β-mercaptoethanol and 0.2 mg protein/ml of bacterial enzymes (GABA-transaminase and succinic semialdehyde dehydrogenase) was added to the droplets and they were incubated at 30 °C for 30 min. After the addition of 0.9 µl of 1.0 N NaOH, the droplets were heated at 60 °C for 20 min and the mixtures were transferred to 45 µl of cycling reagent in 1 ml tubes. The detail of NADPH cycling procedure is described elsewhere[14]. The protein content of ganglia was determined by the method of Lowry et al.[15].

8 distinctive ganglia (abdominal, left (L)- and right (R)-pedal, L- and R-pleural, L- and R-buccal and cervical), connective nerve and buccal muscle were dissected out. The GABA content in each preparation is shown as mmoles/kg protein in the table. GABA was detected in all ganglia and connective nerve. Abdominal ganglion contained the lowest level of GABA among the ganglia examined. The highest level of GABA was found in buccal ganglia, and a fairly high concentration of GABA was also observed in cervical and pedal ganglia.

Concerning the putative transmitters in *Aplysia*, many physiological and pharmacological investigation used the abdominal ganglion and acetylcholine has been found to have both an excitatory and inhibitory action on the postsynaptic membranes[3,16]. In the present investigation, lower concentration of GABA was found in the abdominal ganglion, whereas it was relatively concentrated in the buccal ganglia. In the *Aplysia* ganglia, the level of GABA was 0.3 to 6.2 mmoles/kg protein. If the protein content of the ganglia is assumed to be about $^1/_{10}$ of their wet weight as in other tissues, the values reported here correspond to 0.03–0.62 mmoles/kg wet weight. This value seems to be comparable to the GABA content in mammalian CNS[17]. However, in mammalian spinal cord which contains low concentration of GABA, Miyata et al. found relatively high concentration in the dorsal part of dorsal horn[18]. Although the concentration of GABA in the ganglia is not as high as in the mammalian CNS, GABA can be distributed in the specifically localized areas or neurons of the *Aplysia* ganglia as observed in the lobster ganglion[19].

It should be noted that a relatively high amount of GABA was found in the buccal ganglia. Considering the motor behavior of *Aplysia*, buccal movement seems to be the most striking. It might be worthwhile to study systematically the function of GABA in the *Aplysia* nervous system using the buccal ganglia. Further electrophysiological and pharmacological studies are needed to give light on the role of GABA in the ganglia.

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The levels of metallothionein-like proteins in animal tissues[1]

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Summary. The level of metallothionein-like proteins was determined in different tissues of 6 animal species. The highest concentrations were found in pig and rat tissues. The organs richest in metallothionein-like proteins included: kidneys (101–305 µg/g), intestine (127–257 µg/g) and liver (54–496 µg/g).

Metallothionein and related proteins play a regulatory role in uptake and metabolism of zinc and copper and are of considerable importance in binding heavy metals such as cadmium, mercury and bismuth in animal organs[2]. Estimations of the physiological levels of metallothionein yielded values of 100–260 µg/g for the rat liver and 180–700 µg/g for the rat kidneys[3–8] when using the radiochemical method of estimation of metallothionein[9] or its more specific, modified version[8]. Chen and Ganther[10] obtained values of 42 µg/g and 87 µg/g for the liver and kidneys, respectively, using a method based on molecular filtration with the ^{109}Cd label. Metallothionein levels in